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Impact of a common genetic variation associated with putamen volume on neural mechanisms of ADHD

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Abstract

Objective: In a recent genome-wide association study of subcortical brain volumes,¹ we have identified common genetic variation at rs945270 as having the strongest effect on putamen volume, a brain measure linked to familial risk for attention-deficit/hyperactivity disorder (ADHD). To determine whether rs945270 is a genetic determinant of ADHD, we now explored its impacts on ADHD-related symptoms and on neural mechanisms of ADHD, such as response inhibition and reward sensitivity.

Method: We used a large population sample of 1,834 14-years old adolescents to test the effects of rs945270 on (i) ADHD symptoms accessed through the Strengths and Difficulties Questionnaire (SDQ) and (ii) Region-of-interest (ROI) analyses of putamen activation by functional magnetic resonance imaging (fMRI) using the Stop Signal (SST) and monetary incentive delay (MID) tasks, assessing response inhibition and rewards sensitivity, respectively.

Results: We found a significant link between rs945270 and ADHD symptoms scores, the C-allele being associated with lower symptoms scores, most notably hyperactivity. We also observed sex-specific effects of this variant on the brain. In boys, the C-allele associated with lower putamen activity during successful response inhibition, a brain response that was not associated with ADHD symptoms. In girls, putamen activation during reward anticipation increased with the number of C-alleles, most significantly in the right putamen. Remarkably, right putamen activation during reward anticipation tended to negatively correlate with ADHD symptoms.

Conclusions: Our results indicate that rs945270 may contribute to the genetic risk of ADHD partly through its effects on hyperactivity and reward processing in girls.

Introduction

Abnormalities in subcortical brain structures, notably in the putamen^{2, 3} have been linked to Attention-deficit/hyperactivity disorder (ADHD), a highly heritable and multifactorial neurodevelopmental disorder characterized by symptoms of inattention and hyperactivity/impulsivity,⁴. The age-dependent reductions in putamen volume that normally occur during childhood throughout early adulthood are not observed in ADHD patients and their non-affected siblings,^{5, 6} suggesting that heritable differences in developmental putamen trajectories contribute to the risk for ADHD. Thus, the examination of individual differences in ADHD-related measures including the putamen, in a nonclinical sample of youth may provide valuable insight into the disease.

That abnormalities within the putamen contribute to the pathology of ADHD is further supported findings showing that lesions of the putamen tend to associate with ADHD symptomatology^{7, 8}. Functional neuroimaging approaches have been helpful in identifying the neural mechanisms underlying ADHD symptoms. Task-based fMRI studies have shown that ADHD associates with poor response inhibition (i.e., a deficit in the ability to stop an already planned or initiated action), due to abnormalities in frontal-striatal and frontal-parietal networks in adolescents and young adults with ADHD,⁹⁻¹¹ which may underlie some of the deficits in impulse control observed in ADHD¹². On the other hand, several fMRI studies have observed decreased activation of the ventral striatum – an area including the ventromedial putamen¹³

during anticipation of reward in both adolescents and adults with ADHD, which led to the hypothesis that ADHD is part of a reward deficiency syndrome reflecting the impact of dysregulation of dopamine on the reward system^{14, 15}. Despite evidence implicating the putamen in the pathology of ADHD, its distinctive contributions in the neurobiology of ADHD remains understudied, possibly because, small subcortical brain regions like the putamen are relatively difficult to observe in whole brain fMRI.

Progress to identify genetic factors contributing to putamen development was made through our recent genome-wide meta-analysis that identified several single nucleotide polymorphisms (SNPs) associating with subcortical regions, with the strongest effects observed for rs94527 on the volume of the putamen. Genotypes at this SNP appear to have functional consequences as the C-allele at this locus, which associates with increased putamen volume, also associates with increased expression of the nearby *KTN1* gene in brain and in blood¹. This gene is known to play a role in intracellular organelle motility and it is interesting to note that its expression was found to be decreased in patients with Parkinson's disease¹⁶ and in depressed women undergoing antidepressant-treatment¹⁷. Results of these analyses also indicated that while rs945270 associated with putamen volume across the lifespan, the effects of this SNP on putamen volume tended to decrease with the mean age of each participating cohort¹. This, and the age-dependent reductions of putamen volume from childhood through early adulthood in controls but not in individuals with ADHD or their unaffected siblings⁶, suggested that rs945270's effect on the putamen might predispose to ADHD.

To test this, we analyzed the effects of rs945270 on putamen function and its relation to ADHD-related phenotypes in a large healthy cohort of 14-year-old adolescents, using the Stop-Signal (SST) and the Monetary-Incentive-Delay (MID) fMRI tasks that assess motor control and reward processing, respectively.

Method

Participants

Participants of the study were tested at eight assessment centres (London, Nottingham, Dublin, Mannheim, Berlin, Hamburg, Paris, and Dresden). Local ethics research committees at each site approved the study. On the day of assessment, written consent was obtained from the parent or guardian, and verbal assent was obtained from the adolescent. A detailed description of the recruitment and assessment procedures and inclusion/exclusion criteria have been published elsewhere¹⁸. After quality control, complete and reliable datasets were available from 1129 participants during the reward anticipation phase of the monetary incentive delay task (MID), and from 1227 participants for the successful inhibition in the stop signal task (SST). Demographics information is displayed in Table 1.

ADHD Symptoms (Strength and Difficulties Questionnaire)

ADHD symptoms were assessed using parental reports of the Strength and Difficulties Questionnaire (SDQ), a 25-item behavioral screening tool probing for ADHD-type problems (hyperactivity, inattention, and impulsivity), emotional symptoms, conduct problems, peer problems, and prosocial behavior¹⁹. For this study, we used the SDQ ADHD symptoms scale, which provides a sum score (ranging from 0 to 10) for both hyperactivity/impulsivity (with a 3-items subscale, with scores ranging from 0 to 6) and inattention (with a 2-items subscale, scores ranging from 0 to

4). The Development and Well-Being Assessment (DAWBA)²⁰ was also used to administer a structured interview to parents and children, which was used by experienced clinical raters to assign DSM-IV diagnoses, blind to the SDQ scores.

Genotyping and quality control

DNA purification and genotyping was performed by the Centre National de Génotypage in Paris. DNA was extracted from whole blood samples preserved in ethylene-diamine-tetra-acetic acid (EDTA) vacutainer tubes (BD, Becton, Dickinson and Company, Oxford, United Kingdom) using the Gentra Puregene Blood Kit (QIAGEN, Valencia, California) according to the manufacturer's instructions. Genotype information was collected at 582,982 markers using the Illumina HumanHap610 Genotyping BeadChip (Illumina, San Diego, California)¹⁸. A total of 705 and 1382 individuals were genotyped with the Illumina (Little Chesterford, UK) Human610-Quad Beadchip and Illumina Human660-Quad Beadchip, respectively. Single-nucleotide polymorphisms (SNPs) with call rates <98%, minor allele frequency <1%, or deviation from the Hardy–Weinberg equilibrium ($P < 1 \times 10^{-4}$) were excluded from analyses. Individuals with an ambiguous sex code, excessive missing genotypes (failure rate >2%), and outlying heterozygosity (heterozygosity rate of 3 SDs from the mean) were also excluded. Identity by state similarity was used to estimate cryptic relatedness for each pair of individuals using the PLINK software (pngu.mgh.harvard.edu/~purcell/plink/). Closely related individuals with identity by descent >0.1875 were eliminated from the subsequent

analysis. Population stratification for the GWAS was examined using multi-dimensional scaling (MDS) analysis with HapMap populations as reference groups. Individuals with divergent ancestry (from Utah residents with ancestry from northern and western Europe) were excluded through visual inspection of the first two components. Supplementary Figure S1 shows that our sample is genetically homogeneous (overlapping with the CEU population). The imputation protocol used MaCH software for haplotype phasing and minimac for imputation ²¹.

MRI Data Acquisition and Preprocessing

MRI data were acquired with 3T MRI scanners of different manufacturers (Siemens, Munich, Germany; Philips, Best, The Netherlands; General Electric, Chalfont St Giles, UK; Bruker, Ettlingen, Germany) using the same scanning protocol at all sites (see **SI**). High-resolution T1-weighted 3D structural images were acquired for anatomical localization and coregistration with the functional time series. Forty slices, tilted to the anterior-posterior commissure line, were acquired in descending order (2.4 mm thickness, 1 mm gap) using a gradient-echo T2*-weighted echo-planar imaging (EPI) sequence, and the following parameters: TR = 2200 ms; TE = 30 ms; matrix size 64×64 over a 21.8-cm field of view, giving an in-plane resolution voxel size of 3.4×3.4 mm. For anatomical reference, a 3D gradient echo-based sequence of the whole brain was obtained, using protocol based on the Alzheimer's Disease Neuroimaging Initiative (ADNI) protocol (<http://www.loni.ucla.edu/ADNI/Cores/index.shtml>).

fMRI data were analyzed with SPM8 (Statistical Parametric Mapping, <http://www.fil.ion.ucl.ac.uk/spm>). Spatial preprocessing included: slice time correction to adjust for time differences due to multi-slice imaging acquisition, realignment to the first volume in line, non-linearly warping to the MNI space (based on a custom EPI template (53x63x46 voxels) created out of an average of the mean images of 200 adolescents), resampling at a resolution of 3x3x3mm³ and smoothing with an isotropic Gaussian kernel of 5 mm full-width at half-maximum. At the first level of analysis, changes in the BOLD response for each subject were assessed by linear combinations at the individual subject level, for each experimental condition, each trial (e.g., anticipation of high gain in the MID task) was convolved with the hemodynamic response function to form regressors that account for potential noise variance, e.g. head movement. Estimated movement parameters were added to the design matrix in the form of 18 additional columns (3 translations, 3 rotations, 3 quadratic and 3 cubic translations, and each 3 translations with a shift of ± 1 TR).

We extracted the putamen ROI using the Marsbar toolbox (<http://marsbar.sourceforge.net>), based on the Montreal Neurological Institute Automated Anatomical Labeling. Averaged beta values based on all voxels in the left and right putamen were used for ROI analyses. The current study used the contrast ‘successful stop vs. successful go trials’ to access brain activity associated with inhibition of a motor response in the SST. For the MID task, we used the contrast ‘anticipation of high win vs. anticipation of no win’ based on prior research suggesting reliable associations between ADHD-symptoms and fMRI BOLD

responses measured during reward anticipation ²².

Monetary Incentive Delay Task

To examine neural responses to reward anticipation and outcome, participants performed an age-adapted version of the Monetary Incentive Delay (MID) task ²³ in which sweets instead of money were used as reward. It is a reaction time task that assesses how quickly the subject press a button with left or right index finger to hit a target (white square) that only appears for a short time on the left or right side of the screen.

Stop Signal Task

The stop signal task (SST) was used to study neural responses to successful and unsuccessful inhibitory control ²⁴. Participants were instructed to make a single, button-press response to either of two regularly presented, visual-go stimuli (arrows pointing left or right shown centrally for 1000ms), unless followed by an unpredictable stop signal (arrow pointing upwards shown for 100–300 ms). Twenty percent of the go stimuli were followed by a stop signal. The dependent variable of the task is the stop signal reaction time (SSRT), which was calculated by subtracting the mean stop signal delay (the average time between go and stop signal, at which the subject managed to inhibit to 50% of trials) from the mean reaction time to go trials ²⁵. Because of problems in the tracking algorithm, SSRT data was only available for half of subjects (see **SI**). Participants had first completed a practice session outside the

scanner (for ~5 minutes).

Statistical Analyses

The general linear model was used to calculate associations between ADHD symptom scores, BOLD responses, behavioral measures and genotype, controlling for covariates of sex (depending on the analyses), scanning sites (7 dummy variables) and handedness. All analyses were two-sided. Since those hypotheses for brain activity involved the two hemispheres and two contrasts, the significance threshold was set to 0.0125 using Bonferroni correction.

To investigate the stability of our main findings, we have carried out two internal replications. First, we tested the same association in eight research sites separately. The regression coefficients (β) and their corresponding Standard Errors (SEs) were then used to conduct a meta-analysis. The R package *rmeta* (<https://cran.r-project.org/web/packages/rmeta/rmeta.pdf>) was then used to generate the forest plot. Second, a resampling of the original data with replacement (a bootstrapping process) was conducted to generate a new sample with the same sample size as the original data. The process was replicated 10000 times, and all of the *t* statistics were used to calculate a bootstrapping *p*-value. By these two replications, we demonstrated both the stability of the results and the fact that they are unlikely to reflect sample specific variance.

Results

Relationship of rs945270 with ADHD symptoms

In our population cohort of 14-year-old adolescents, each copy of the C allele was associated with a 77.4 mm³ increase in the averaged volume of the bilateral putamen ($\beta = 77.4$, 1.1% variance explained, $p = 1.3 \times 10^{-5}$). While we noted a sex difference for putamen volume (smaller volume in girls than in boys: $\beta = -554.5$, $p < 10^{-10}$), there were no interaction between sex and genotype at rs945270 on putamen volume ($\beta = -16.1$, $p = 0.65$). Investigating the relationship between rs945270 and ADHD symptoms, we found that rs945270 significantly associated with ADHD symptoms in the full sample. The C-allele at rs945270 associated with lower scores for ADHD symptoms compared to the G-allele, such that each C-allele is expected to reduce symptoms scores by 0.067 standard deviation ($\beta = -0.21$, $\beta_{\text{stand}} = 0.067$, $p = 0.0053$; bootstrapping: $p = 0.0070$; meta-analysis: $\beta = -0.21$, $p = 0.0063$; Figure 1 and Table 2). Testing for the specificity of rs945270 on ADHD symptoms, we found that variation at rs945270 was mostly associated with hyperactivity symptoms ($\beta = -0.126$, $\beta_{\text{stand}} = -0.065$, $p = 0.0054$ and $\beta = -0.093$, $\beta_{\text{stand}} = -0.051$, $p = 0.028$ for the hyperactivity and inattention subscales, respectively). Testing for possible sex differences in ADHD symptoms, we found that girls scored lower for ADHD symptoms than boys ($\beta = -0.77$, $\beta_{\text{stand}} = -0.169$, $p = 1.12 \times 10^{-12}$), differing relatively more in inattentive ($\beta = -0.406$, $\beta_{\text{stand}} = -0.162$, $p = 6.77 \times 10^{-12}$) than in hyperactive ($\beta = -0.361$, $\beta_{\text{stand}} = -0.134$, $p = 1.37 \times 10^{-8}$) symptoms. There was no sex-by-rs945270 genotype interaction on ADHD symptoms ($\beta = 0.049$, $p = 0.71$). ADHD

symptoms scores were not associated with putamen volume in this sample ($\beta = -0.00012$, $p = 0.28$); the association between rs945270 genotypes and ADHD symptoms remained significant after controlling for the whole putamen volume.

Testing for the specificity of rs945270 effects, we analyzed its association with the other four symptoms domains measured by the SDQ. We found nominal association of rs945270 with the emotional symptoms score ($\beta = -0.15$, $p = 0.021$), not with the conduct ($\beta = -0.048$, $p = 0.37$), peer problem ($\beta = -0.074$, $p = 0.17$) and prosocial ($\beta = 0.075$, $p = 0.21$) scores.

As about 2.5% of our sample had received a diagnosis of ADHD (11 girls and 35 boys), based on the DAWBA²⁰, we tested how well the SDQ symptoms scores correlated with the ADHD diagnosis. There was a moderate positive correlation ($\beta = 0.304$, $p = 4.23 \times 10^{-40}$) between the two variables, confirming the utility of the SDQ as a screening tool for ADHD in the community²⁶.

Response Inhibition

We next tested the effects of rs945270 on inhibitory control, assessing blood-oxygen-level dependent (BOLD) responses in the putamen during successful inhibition of motor response in the SST fMRI task. The C-allele of rs945270 associated with decreased putamen activity (left putamen: $\beta = -0.088$, $p = 0.0030$; bootstrapping $p = 0.0060$; meta-analysis, $\beta = -0.080$, $p = 0.0039$; right putamen: $\beta = -0.089$, $p = 0.0018$, bootstrapping $p = 0.0020$; meta-analysis, $\beta = -0.094$, $p = 0.0016$) in the full sample (Table 3). Girls had lower BOLD-responses than boys, most

significantly in the left putamen (left: $\beta = -0.15$, $p = 0.002$; right: $\beta = -0.084$, $p = 0.035$), and we observed a significant sex-by-genotype interaction in the left ($\beta = 0.16$, $p = 0.0080$; bootstrapping $p = 0.0080$; meta-analysis $\beta = 0.17$, $p = 0.013$), but not in the right putamen ($\beta = 0.087$, $p = 0.12$) (Table 3). Sex-specific analyses indicated that the associations of rs945270 with putamen activity observed in the full sample were mainly driven by boys; the C-allele was associated with decreased putamen activation in this group (left putamen: $\beta = -0.16$, $p = 0.0005$; right putamen: $\beta = -0.13$, $p = 0.004$), but not in girls (left putamen: $\beta = -0.010$, $p = 0.78$; right putamen: $\beta = -0.047$, $p = 0.20$) (Figure 2A,B). To test if these effects of rs945270 on putamen activation in boys were localized rather than distributed throughout the whole putamen, we performed post-hoc, voxel-wise association within the putamen. These revealed significant clusters of activity only in the right putamen, in caudal regions (Supplementary Figure S2, Table S1). This suggests that rs945270 influences response inhibition in boys throughout global rather than localized effects on the left putamen. Although we observed significant negative association between putamen volume and left putamen activation in the full sample (left: $\beta = -0.000093$, $p = 0.018$, right: $\beta = -0.000063$, $p = 0.12$), the association between genotype and putamen activation remained significant after controlling for putamen volume. However, while rs945270 variation associated with putamen activation during successful inhibition in boys, we found no significant association between putamen activation and ADHD symptoms, neither in the full sample (Table 3) nor when analyzing boys only (left: $\beta = 0.16$, $p = 0.19$, right: $\beta = 0.10$, $p = 0.43$). This indicates differences in putamen

activation during successful inhibition are unlikely to account for the association between rs945270 and ADHD symptoms noted above.

To test if these differences in putamen activation were reflected at the behavioural level, we analyzed a subsample of $N = 671$ individuals with information on duration of the stop process (SSRT), a known proxy for ADHD symptoms that also correlated with ADHD symptoms scores in our sample ($\beta = 1.44, p = 0.014$). Putamen activation during successful stopping negatively correlated with SSRT in the full sample (left: $\beta = -12.28, p = 2.50 \times 10^{-8}$, right: $\beta = -11.83, p = 1.0 \times 10^{-6}$), so that individuals who stopped their responses more quickly activated this region more strongly. While there were no sex differences for SSRT ($\beta = -1.532, p = 0.68$), an interaction between sex and left putamen activation on SSRT ($p = 0.009$) was observed. Left putamen activation during response inhibition negatively correlated with SSRT in boys, not in girls (boys: $\beta = -16.97, \beta_{\text{stand}} = -0.309, p = 10^{-10}$; girls: $\beta = -5.52, \beta_{\text{stand}} = -0.112, p = 0.090$).

Reward anticipation

In the fMRI reward anticipation task, we observed lesser activation in the putamen bilaterally in girls compared to boys (left: $\beta = -0.10, p = 5 \times 10^{-6}$; right: $\beta = -0.090, p = 3.9 \times 10^{-5}$). No significant main effect of rs945270 genotype on putamen activation was observed ($\beta = 0.016, p = 0.66$ and $\beta = 0.022, p = 0.38$, for the left and right putamen, respectively). However, there was a significant sex-by-genotype interaction for activation in the right putamen ($\beta = 0.084, p = 0.0065$; bootstrapping $p = 0.011$;

meta-analysis, $\beta = 0.065$, $p = 0.0044$; Left putamen: $\beta = 0.054$, $p = 0.086$; Figure 3A,B). Sex specific analyses indicated that putamen activation increased with the number of C-alleles at rs945270 in girls, most significantly in the right putamen (left: $\beta = 0.045$, $p = 0.035$; right: $\beta = 0.066$, $p = 0.002$). We performed post-hoc voxel-wise association analysis within the putamen to test if these effects of rs945270 on right putamen activation in girls were due to localized effects of rs945270 in the putamen. These identified significant clusters in the right anterior ventral putamen associating with rs945270 (Supplemental Figure S3, Supplemental Table S2). This indicates that rs945270 influences reward sensitivity in girls through localized effects on the right putamen. Analyses in boys did not reveal genotype differences (left: $\beta = -0.009$, $p = 0.71$, right: $\beta = -0.019$, $p = 0.4$). There were significant associations between putamen volume and putamen activation in this task (left: $\beta = 0.000085$, $p = 0.000041$, right: $\beta = 0.000078$, $p = 0.00023$), which were driven by girls (left: $\beta = 0.00012$, $p = 0.000016$, right: $\beta = 0.00011$, $p = 0.00007$), not by boys. Besides, the associations between genotype and putamen activation remained significant after controlling for putamen volume.

Given the reported decrease in ventral striatal activation during anticipation of reward in both adolescents and adults with ADHD²⁷⁻³⁰, we investigated this link further in our sample. We found significant negative association between right putamen activation during reward anticipation and ADHD symptoms scores in the full sample (left: $\beta = -0.009$, $p = 0.089$, right: $\beta = -0.012$, $p = 0.014$). We found a negative trend for association between right putamen activation during reward

anticipation and ADHD symptoms in the full sample (left: $\beta = -0.009$, $p = 0.089$, right: $\beta = -0.012$, $p = 0.014$), reflecting an association with hyperactivity rather than inattention (Table 3). This suggests that the G-allele at rs945270 may partly contribute to the risk of ADHD through its negative effects on reward processing and positive effect on hyperactivity, particularly in girls.

Discussion

Our study provides the first genetic link between putamen structure and function and risk for ADHD. We demonstrate that the rs945270 variant, identified in a previous genome-wide meta-analysis of putamen volume, associates with ADHD symptoms scores at age 14 years, notably with hyperactivity. We also found it may contribute to the genetic risk of ADHD by influencing neural mechanisms in a sex-specific manner. Specifically, the G-allele at this locus may partly contribute to the risk of ADHD in girls through its negative effects on reward processing in the right anterior ventral putamen and positive effect on hyperactivity. We also found that rs945270 genotypes affected putamen activation during successful response inhibition in boys, an effect that did not correlate with ADHD symptoms.

Our findings that a genetic polymorphism influences neural mechanisms related to ADHD (i.e., reward sensitivity) confirms previous evidence suggesting that a common set of genes may contribute to the distinct symptoms of ADHD³¹. They also considerably extend these conclusions by providing a genetic mechanism that clarifies sexually dimorphic phenomena associated with ADHD³². Sexual dimorphisms were evident during both response inhibition and reward anticipation, the putamen being activated to a lesser extent in girls compared to boys. The G-allele at rs945270 not only associated with higher scores of ADHD symptoms, it also accentuated sexually dimorphic responses by increasing putamen activity during response inhibition in boys and decreasing that in girls during reward anticipation. That girls carriers of the

risk G-allele at rs945270 exhibit significantly lowered putamen activation compared to carriers of the C-allele when anticipating a reward, suggests that this locus influences reward sensitivity in girls. The negative correlation between BOLD responses in the right putamen during reward anticipation and ADHD symptoms is indeed consistent with a blunted reward system,²⁹ as evidenced by hypo-responsiveness of the ventral striatum (VS) in ADHD patients^{27, 28, 33, 34}.

Our voxel-wise analyses showing specific effects of rs945270 on the anterior and the posterior putamen in the reward- and motor response-related tasks, respectively provide further support earlier reports of putamen segmentation³⁵⁻³⁸ indicating gradual functional specialization within the putamen. Rostral subregions are important for goal-direct behaviours, motivation and emotional processing, and higher-order cognitive aspects of motor function, such as learning new movements, through connections to the anterior cingulate cortex, pre-supplementary motor area, and prefrontal cortex³⁹. In contrast, more caudal regions are implicated in planning and execution of well-learned, skilled movements, through connections to primary and supplementary motor cortices^{39, 40}. A major implication of our study is that genes may impact these putamen-related functions differently in men and women.

Our use of parental reports for the SDQ have a number of limitations. The SDQ has demonstrated reasonable efficiency in screening for ADHD in the community²⁶ and it is notable that its hyperactivity/inattention scale performs similarly to the ‘any

disorder' scale despite its brevity. However, this screening efficiency depends on the informant, with the combination of parents and teachers reports being a better predictor of ADHD. Thus, our use of single informant limited our ability to detect ADHD symptoms. It has also limited our ability to properly measure the impact of emotional disorders, which are better captured by self-reports. These limitations are further exacerbated by the poor ability of the SDQ to identify other disorders such as specific phobias, panic disorder/agoraphobia, eating disorders and separation anxiety. Thus, comorbidity and other unmeasured covariates, such as socio-economic status, parental psychopathology and life events could have contributed to our findings.

Nonetheless our findings illustrate the sex-dependant impact of a genetic variation on human brain function and potential implication for ADHD. Further studies of patients and their controls, taking the above limitations into account, are needed to determine if rs945270 can help the detection and classification of ADHD and its subtypes and disease outcome.

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Table 1: Demographics split by tasks (MID and SST) and sex. Means, standard deviations are presented below (Mean \pm SD).

	Reward Anticipation		Successful inhibition	
	Boys (n = 543)	Girls (n = 586)	Boys (n = 577)	Girls (n = 650)
Age (in years)	14.4 \pm 0.4	14.4 \pm 0.4	14.4 \pm 0.4	14.4 \pm 0.4
Handedness*	84 L/A 459 R	62 L/A 524 R	78 L/A 499 R	62 L/A 588 R
rs945270	GG: 103	GG: 97	GG: 107	GG: 106
genotypes	GC: 229	GC: 302	GC: 267	GC: 336
	CC: 211	CC: 187	CC: 203	CC: 208
ADHD	3.2 \pm 2.2	2.4 \pm 2.1	3.1 \pm 2.3	2.4 \pm 2.1
Symptoms				
scores				

L/A: Left-handed or ambidextrous, R: Right-handed.

Table 2: Associations of rs945270 and sex with ADHD symptoms.

	rs945270 (effect C-allele)	Sex	Sex * rs945270
ADHD symptoms	$\beta = -0.21$, $p = 0.005$	$\beta = -0.77$, $p = 1.12 \times 10^{-12}$	$\beta = 0.049$, $p = 0.71$
Hyperactivity	$\beta = -0.126$ $p = 0.005$	$\beta = -0.361$ $p = 1.37 \times 10^{-8}$	na
Inattention	$\beta = -0.093$ $p = 0.028^*$	$\beta = -0.406$ $p = 6.77 \times 10^{-12}$	na

Associations significant affect Bonferroni correction for multiple testing are in bold. *, nominally significant association; na, not assessed.

Table 3: Associations of rs945270, sex on ADHD symptoms on putamen activation split by tasks (SST and MID).

	SST		MID	
	Left putamen	Right putamen	Left putamen	Right putamen
rs945270 (effect C-allele)	$\beta = -0.088$, $p = 0.0030$	$\beta = -0.089$ $p = 0.0018$	$\beta = 0.016$, $p = 0.66$	$\beta = 0.022$, $p = 0.38$
Sex	$\beta = -0.15$, $p = 0.002$	$\beta = -0.084$, $p = 0.035$	$\beta = -0.10$, $p = 5 \times 10^{-6}$	$\beta = -0.090$, $p = 3.9 \times 10^{-5}$
Sex * rs945270	$\beta = 0.16$, $p = 0.0080$	$\beta = 0.087$, $p = 0.12$	$\beta = 0.054$, $p = 0.086$	$\beta = 0.084$, $p = 0.0065$
ADHD symptoms	$\beta = 0.002$ $p = 0.80$	$\beta = 0.003$ $p = 0.76$	$\beta = -0.009$, $p = 0.089$	$\beta = -0.012$, $p = 0.014^*$

Hyperactivity	$\beta = 0.010$ $p = 0.52$	$\beta = 0.008$ $p = 0.60$	$\beta = -0.015$, $p = 0.072$	$\beta = -0.021$, $p = 0.013^*$
Inattention	$\beta = -0.004$ $p = 0.83$	$\beta = -6.4 \times 10^{-5}$ $p = 0.99$	$\beta = -0.011$, $p = 0.25$	$\beta = -0.016$, $p = 0.076$
Sex * ADHD	$\beta = -0.036$, $p = 0.06$	$\beta = -0.02$, $p = 0.27$	$\beta = 0.008$, $p = 0.45$	$\beta = -0.006$, $p = 0.55$
Sex * Hyperactivity	$\beta = -0.033$, $p = 0.30$	$\beta = -0.014$, $p = 0.64$	$\beta = 0.006$, $p = 0.73$	$\beta = 0.008$, $p = 0.64$
Sex * Inattention	$\beta = -0.075$, $p = 0.028^*$	$\beta = -0.47$, $p = 0.15$	$\beta = 0.018$, $p = 0.34$	$\beta = -0.01$, $p = 0.59$

Associations significant affect Bonferroni correction for multiple testing are in bold. *, nominally significant associations.

Figures

Figure 1: A) Relationship between ADHD symptoms scores and rs945270 in 14 year-old adolescents (error bars denote SEM). B) Forest plots of associations between ADHD symptoms scores and rs945270 in the 8 research sites and meta-analysis. The β values obtained at each research site are shown as black squares, and the ranges between upper and lower CI95s are shown as the black lines. The integrated β as well as its upper and lower CI95s were calculated from the meta-analysis by using the inversion of squared SEs as weight, and the result is illustrated as the black diamond, where the mean is indicated vertically and the range between upper and lower CI95s is indicated horizontally.

Figure 2: Relationship between rs945270 genotypes and BOLD responses within the left (A) and right (B) putamen during successful inhibition of motor response in the SST fMRI task (blue and red represent boys and girls, respectively, error bars denote SEM).

Figure 3: BOLD responses within the left (A) and right (B) putamen during reward anticipation in the MID fMRI task stratified by rs945270 genotypes (blue and red represent boys and girls, respectively, error bars denote SEM).